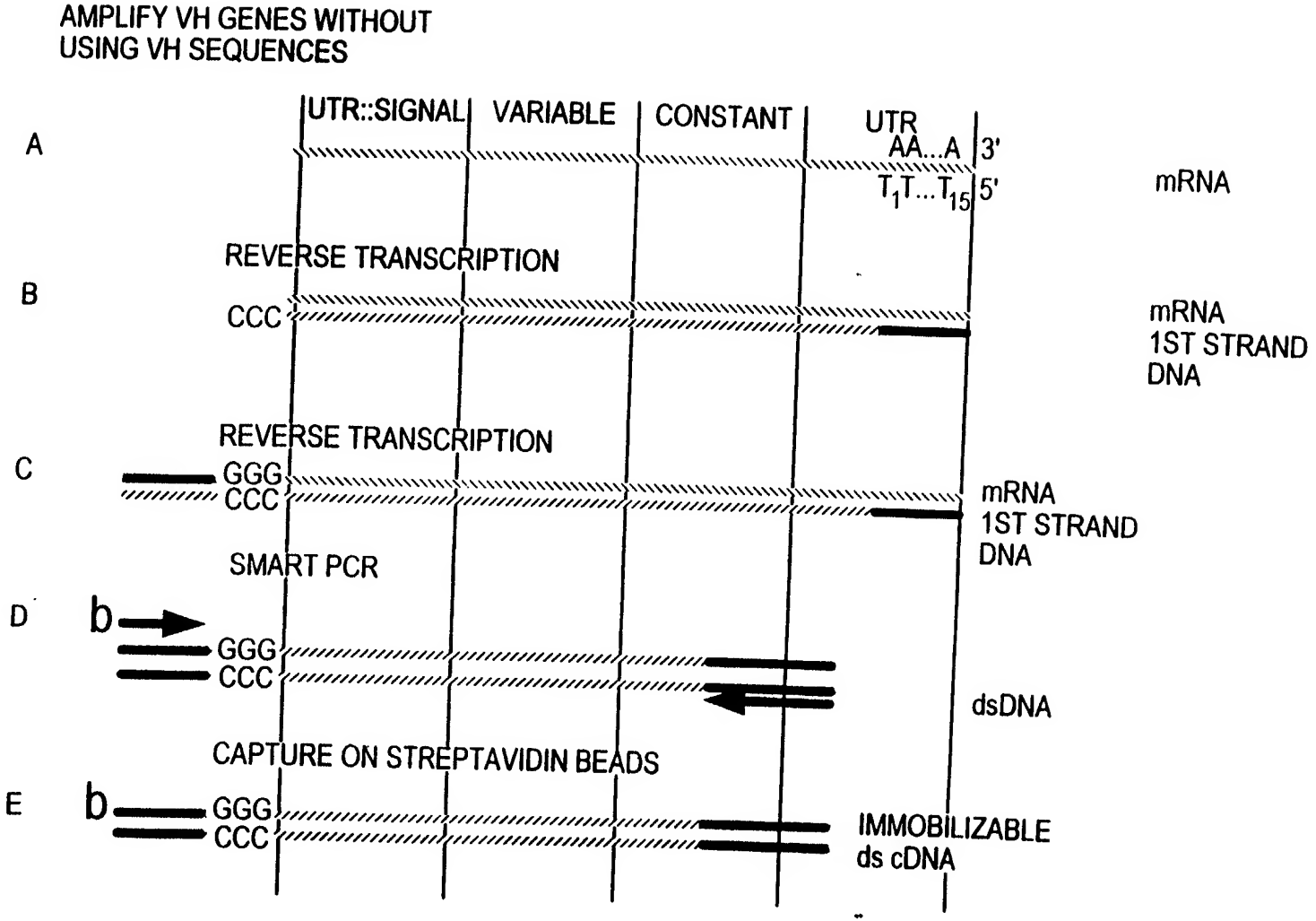


FIG. 1

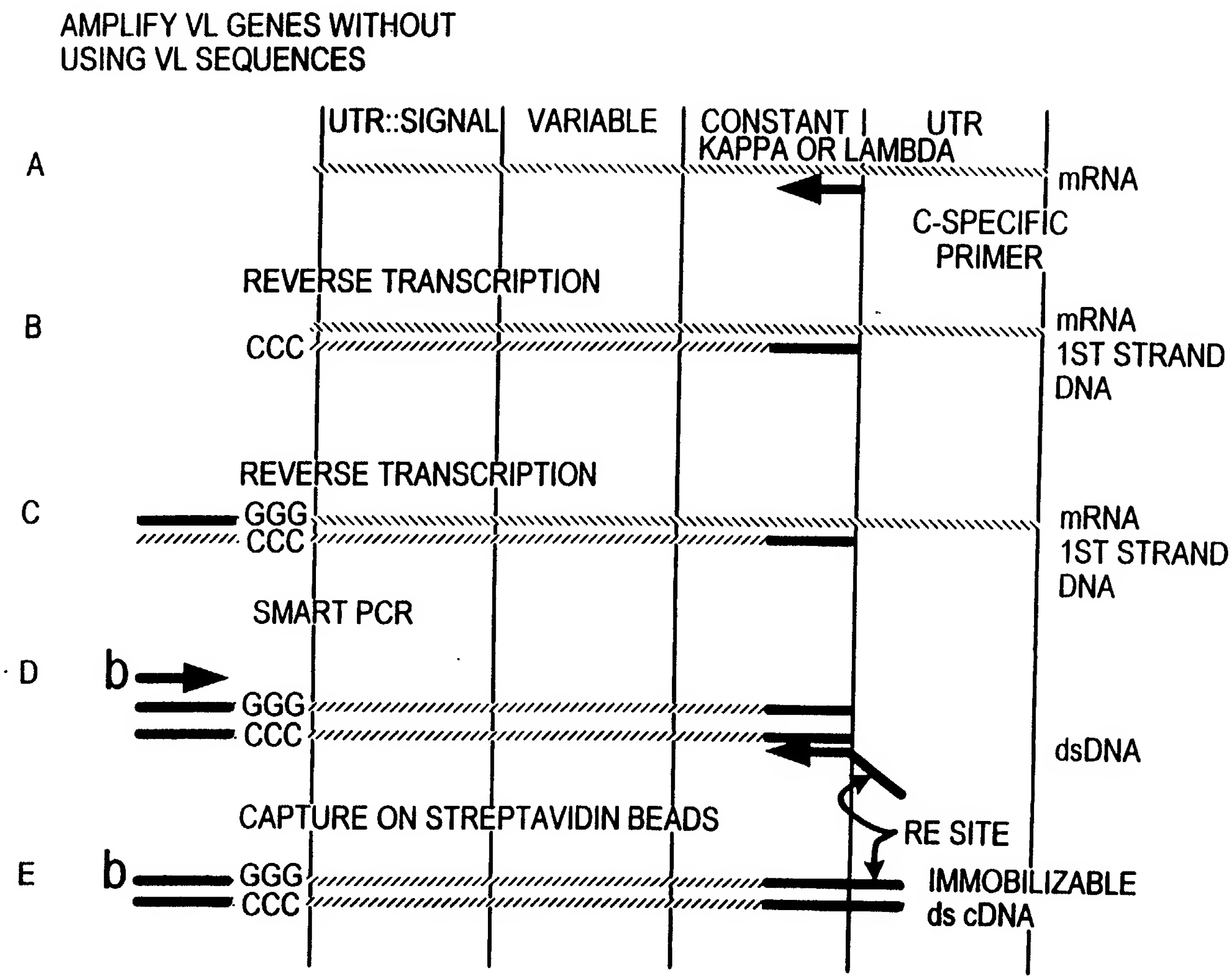


FIG. 2

RACE non-biased antibody V-gene amplification

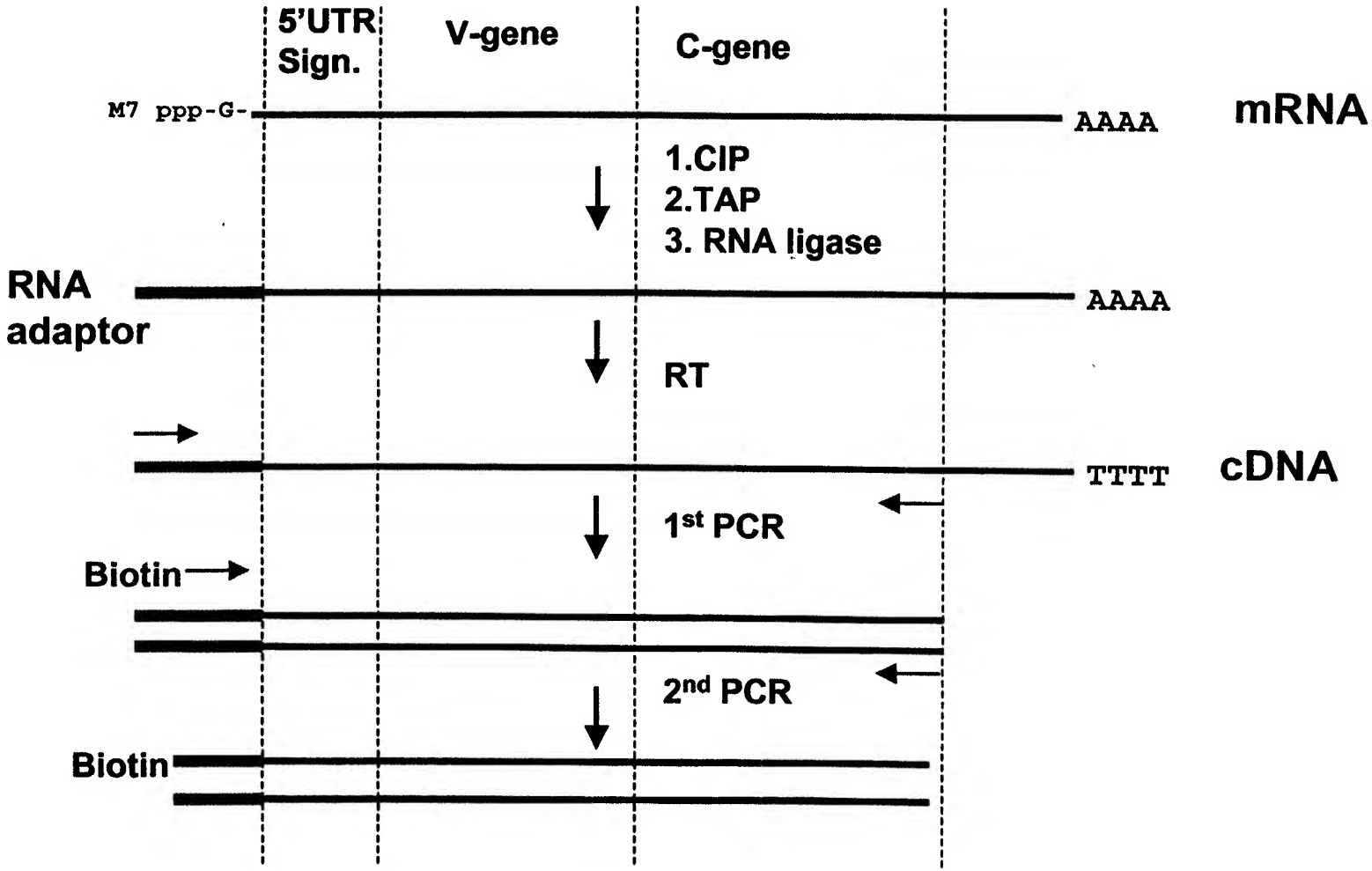
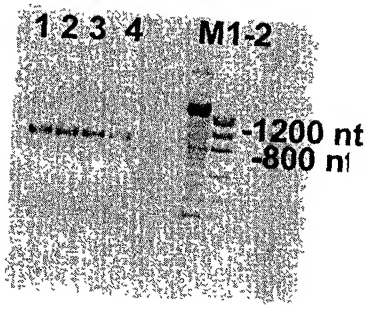


FIG. 3

1, 2, 3 and 4 are patient samples

1st PCR heavy chains



1st PCR light chains

Kappa

Lambda

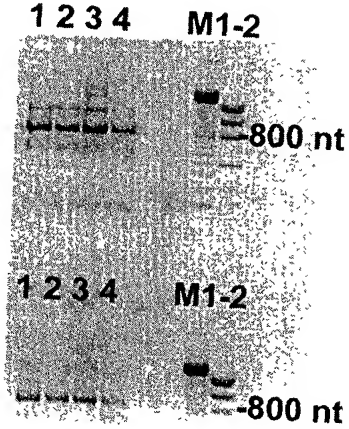
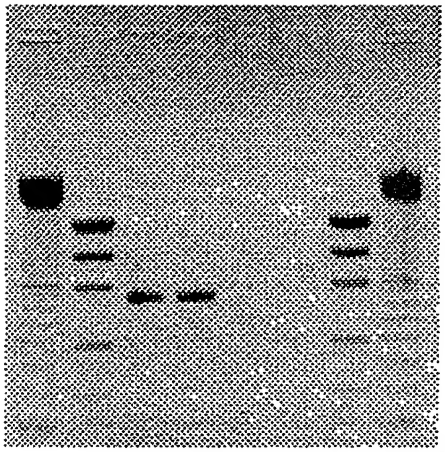


FIG. 4

1 2 3 4 5 6 7 8



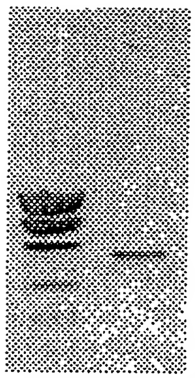
Gel analysis of PCR product from extender-kappa amplification

Approx. 75ng/5µl → 15ng/µl

1 - 100bp
2 - LDM
3 - 50ng template
4 - 10ng template
5 - ssDNA unligated
6 - negative control
7 - LDM
8 - 100bp

FIG. 5

1 2



Gel purified PCR product from extender-kappa amplification

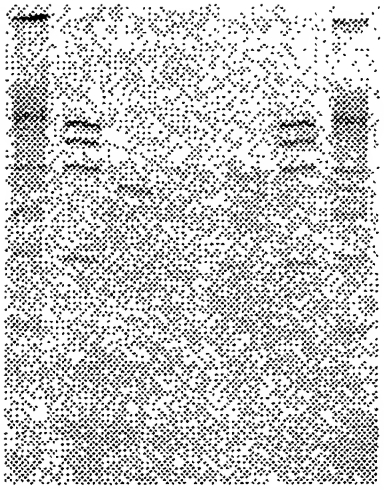
Concentration : ± 35ng/µl

1 - LDM

2 - 1µl purif.

FIG. 6

1 2 3 4 5 6 7



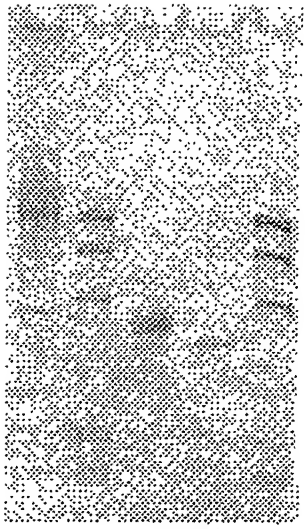
Gel-analysis of digested κ-ssDNA

1µl digested ssDNA ≈ 8ng ssDNA
Total volume of 50µl = 400ng ssDNA
➔ 400ng ssDNA available for ligation of the bridge-extenders

1 - 100bp
2 - LDM
3 - 1µl ssDNA pure
4 - 4µl beads after dig.
5 - 8µl beads after dig.
6 - LDM
7 - 100bp

FIG. 7

1 2 3 4 5



Gel analysis of extender – cleaved kappa ligation

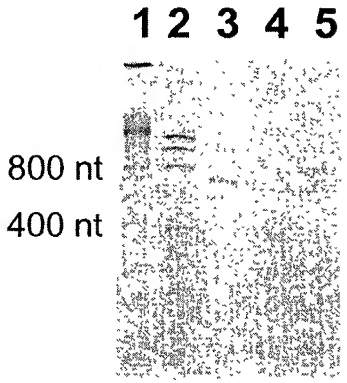
20ng/5µl eluted material → 4ng/µl

1- 100bp
2 - LDM
3 - Ligationmix, 4µl
4 - Unligated ssDNA
5 - LDM

FIG. 8

Cleavage and ligation Kappa light chains

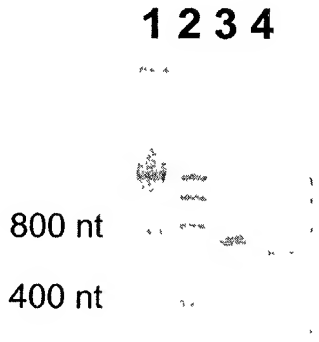
A) BsmA1 cleavage



1. 100 bp marker
2. LDM marker
3. Sup. ssDNA after dig.
4. beads after dig. (uncleaved material)
5. DNA before cleavage

80% cleavage

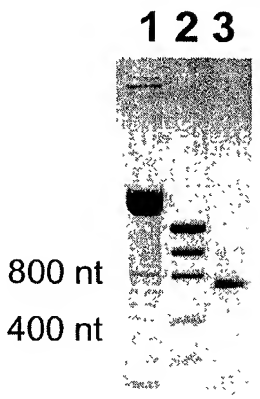
B) Bridge Ligation



1. 100 bp marker
2. LDM marker
3. Ligationmix
4. Unligated ssDNA

90% ligation

C) PCR



1. 100 bp marker
2. LDM marker
3. 50 ng template (13 cycles)

FIG. 9

VH-CDR1
1 Y 1 M 1

VH-CDR2
2 I 2 3 S G G 1 T 1 YADSVKG

FIG. 10

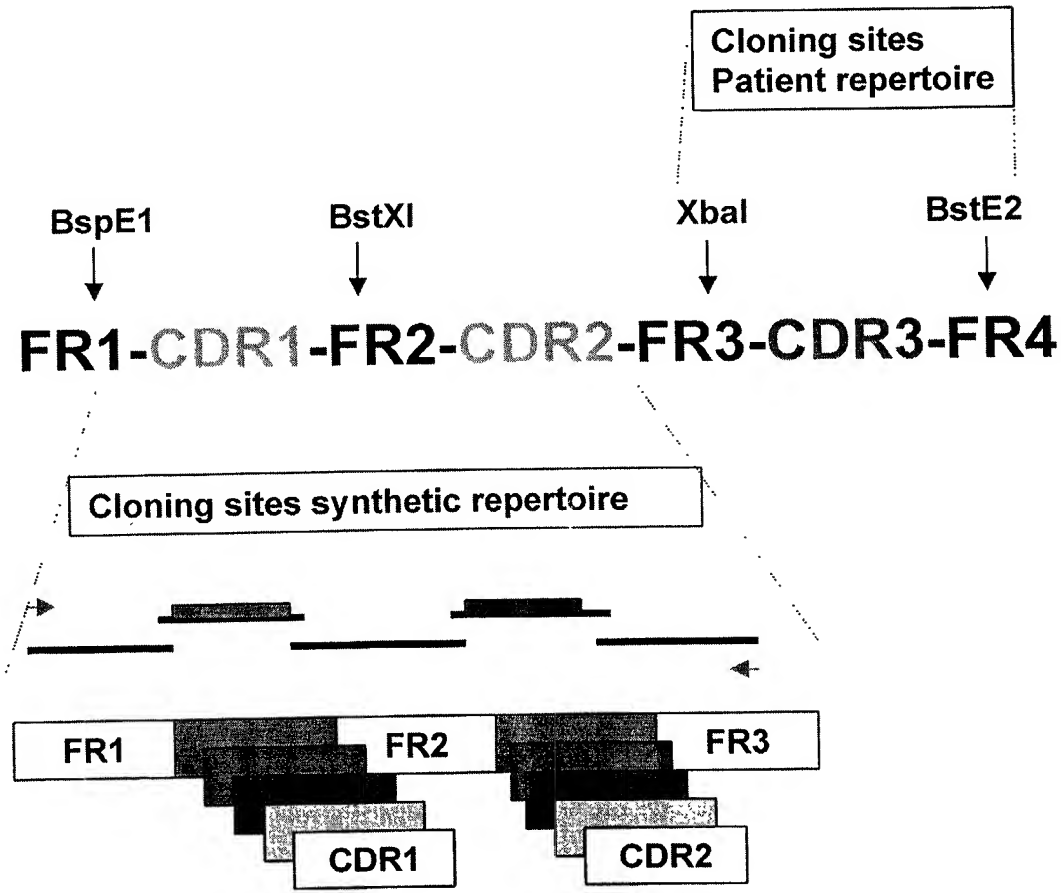


FIG. 11

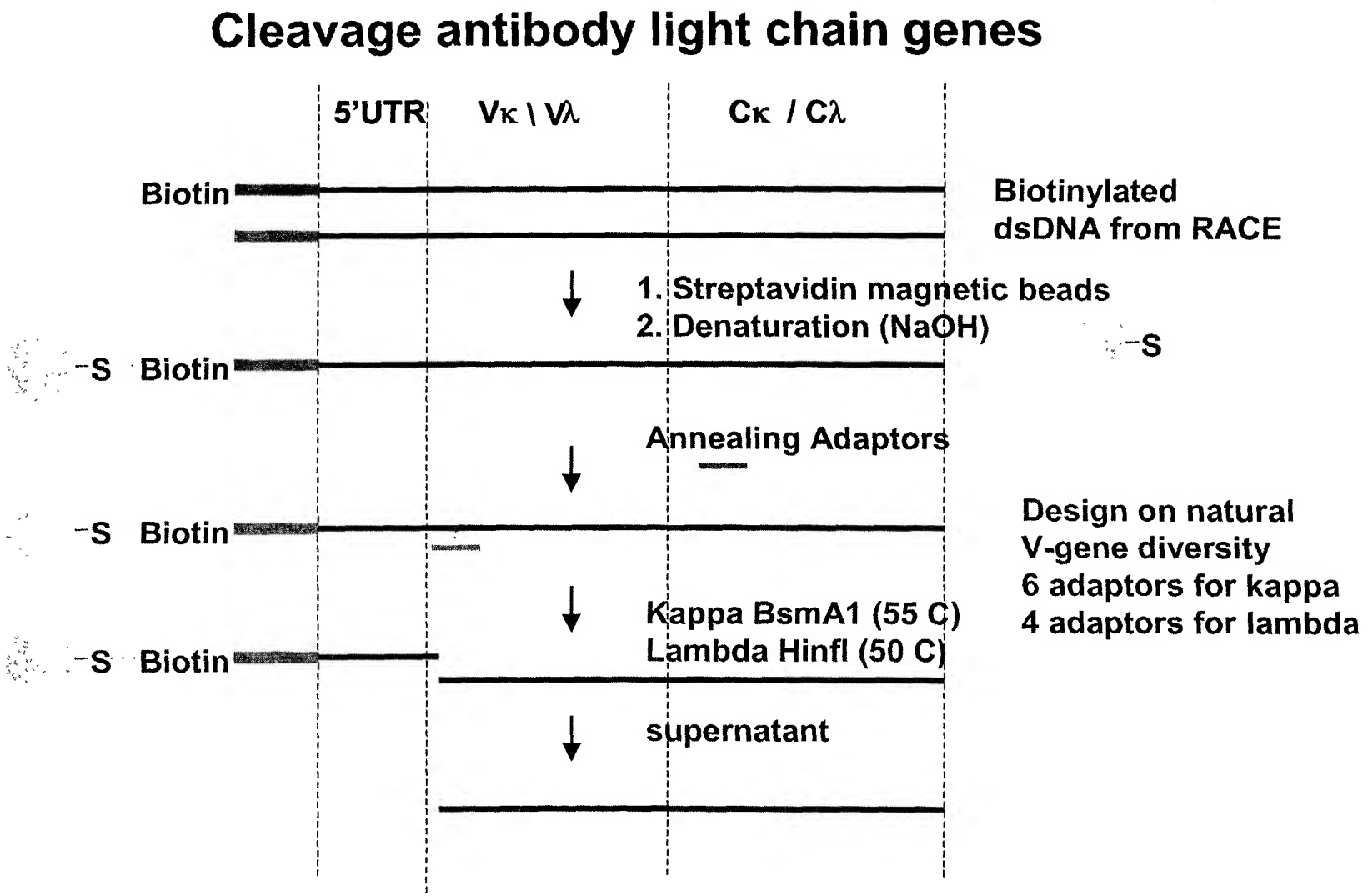


FIG. 12A

Ligation of cleaved light chains

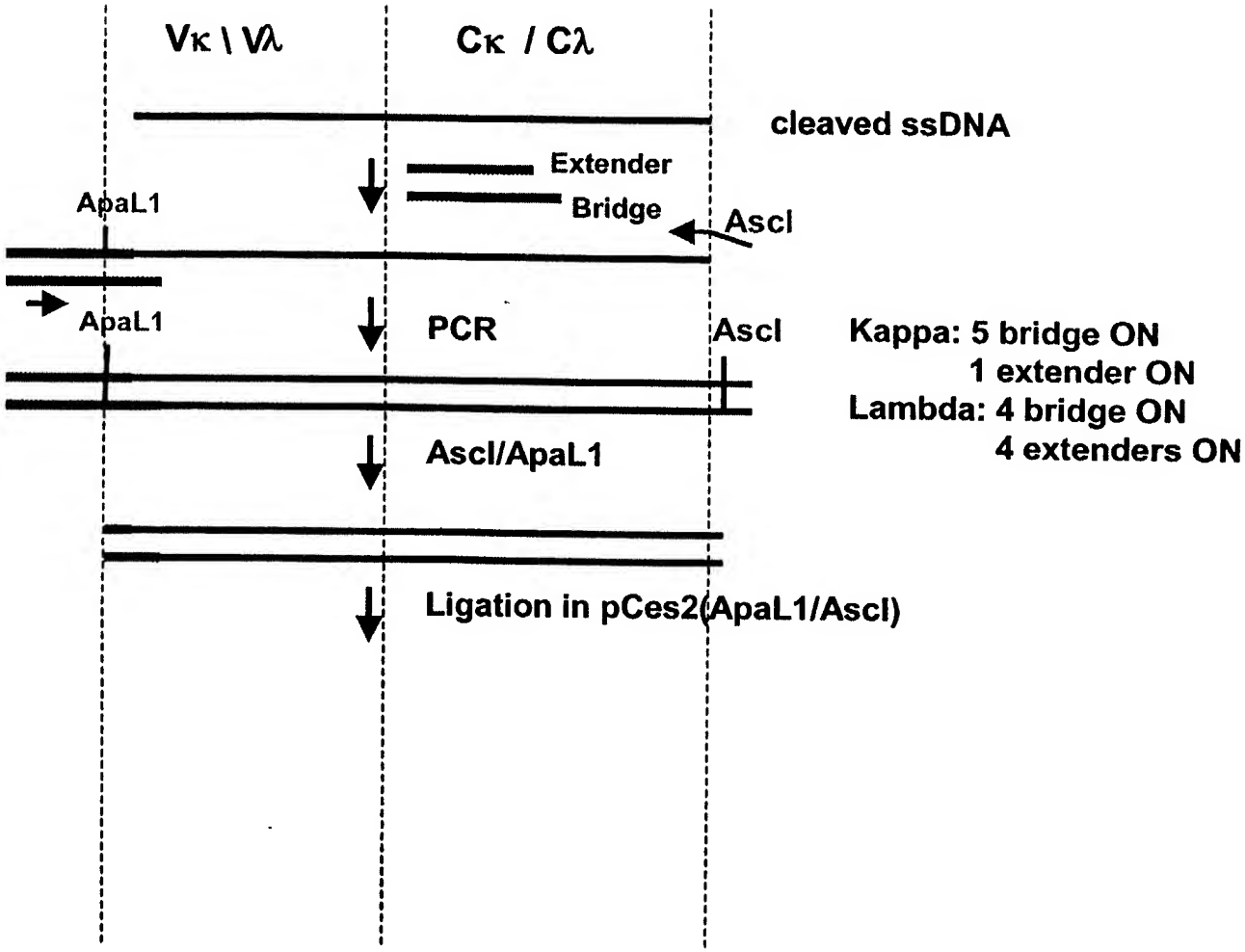
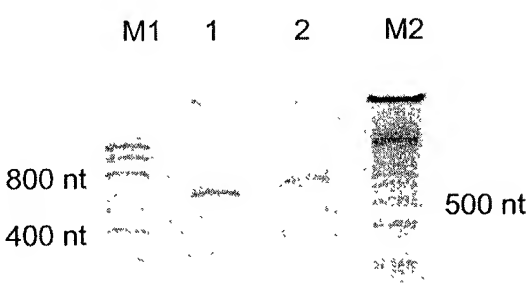


FIG. 12B

Figure 3: Cleavage and ligation lambda light chains

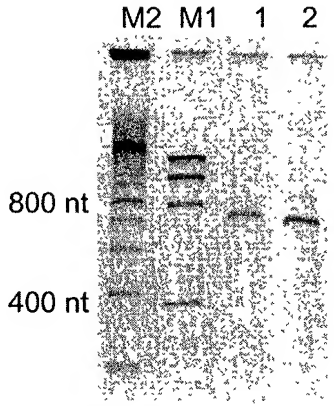
A) HinfI cleavage



M1 = LDM MW marker
1 = sup. after Hinf-cleavage (10ng ssDNA)
2 = beads after Hinf-cleavage (10ng ssDNA (not cleaved)
M2 = 100bp marker , bottom to top: 300nt, 400nt, 500nt, 600nt, 800nt

70% cleavage

B) Bridge ligation



1=Ligated DNA
2= Unligated DNA

95% ligation

C) PCR

1= 1ul purified PCR product / 50 ul (15 cycles)

FIG. 13

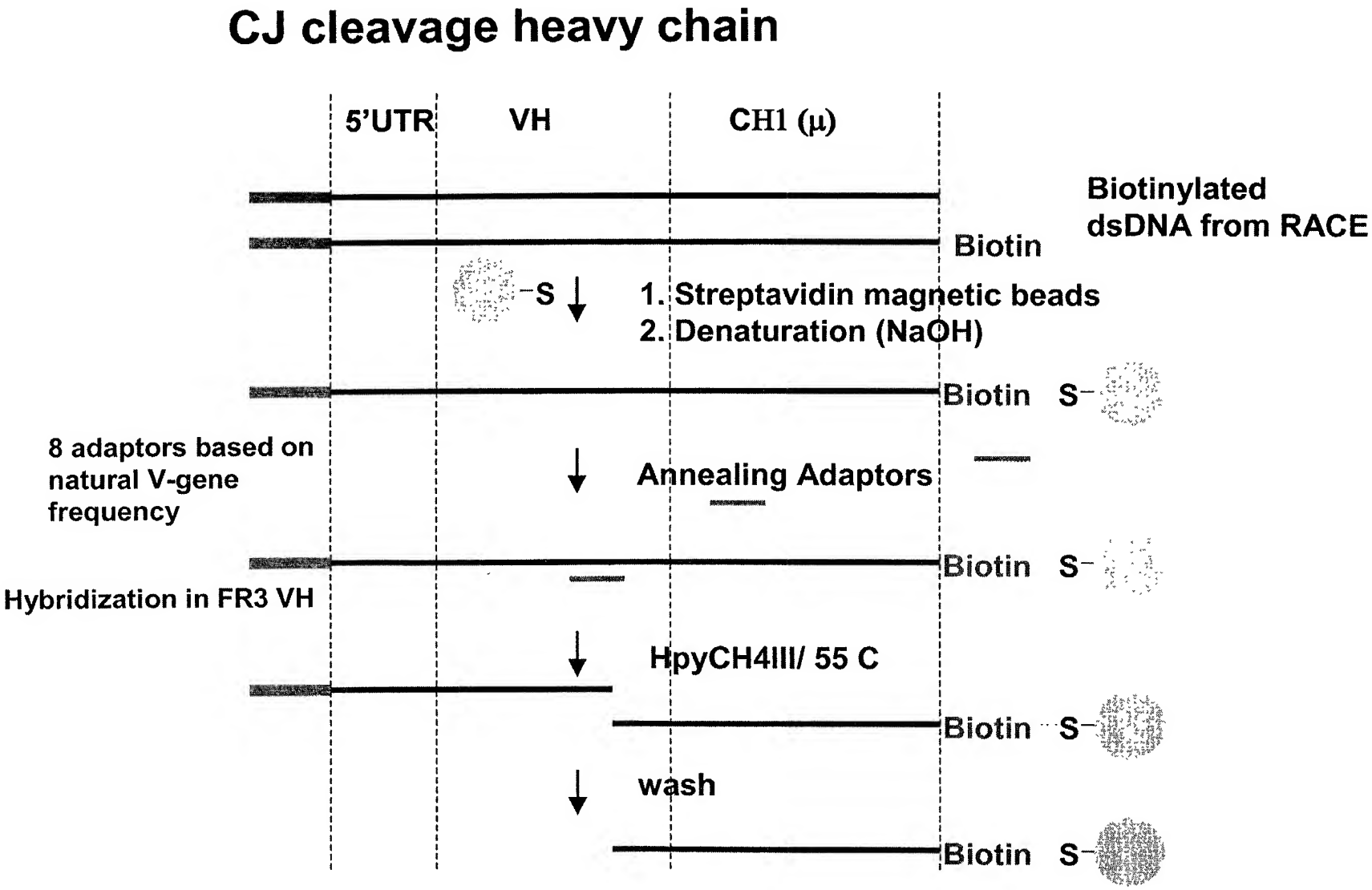


FIG.14A

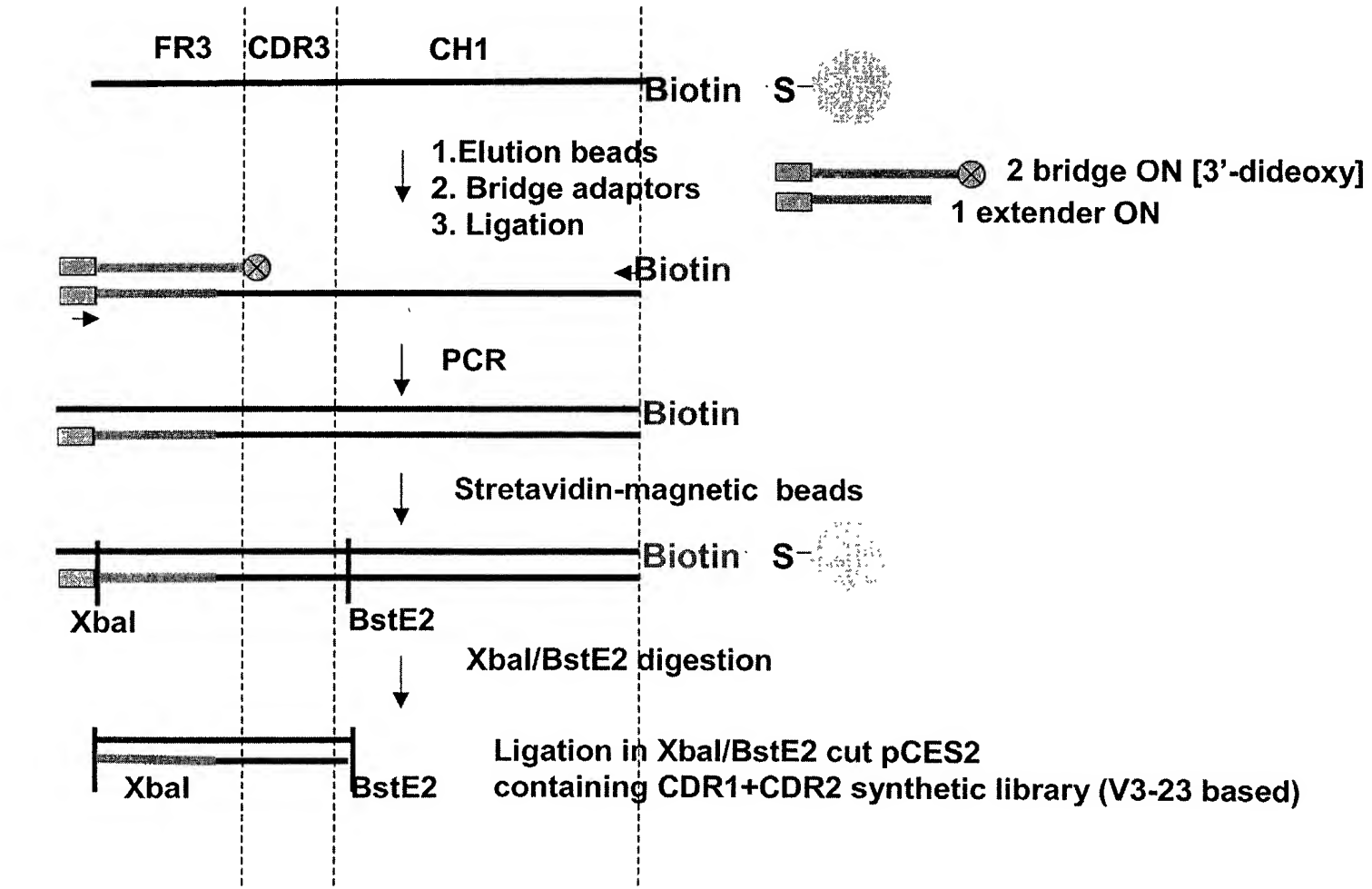
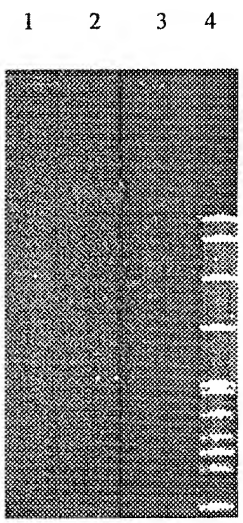

Ligation heavy chain CDR3 diversity
FR3
CDR3
CH1
Biotin
S
1.Elution beads
2. Bridge adaptors
3. Ligation
2 bridge ON [3'-dideoxy]
1 extender ON
Biotin
PCR
Biotin
Stretavidin-magnetic beads
Biotin
S
XbaI
BstE2
XbaI/BstE2 digestion
XbaI
BstE2
Ligation in XbaI/BstE2 cut pCES2
containing CDR1+CDR2 synthetic library (V3-23 based)

FIG. 14B

Cleavage and ligation Heavy Chain

A) HpyCH4III cleavage



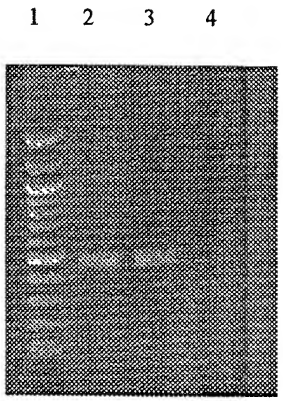
1 = Cleaved DNA cluted from PN column

2 = Beads after HpyCH4III digestion

3 = Supernatant after cleavage

4 = MspI digest of pBR322

B) PCR



1 = NEB 100bp ladder

2 = 5ul/100ul PCR product 20 cycles; sample A

3 = 5ul/100ul PCR product 20 cycles; sample B

4 = no template

FIG. 15

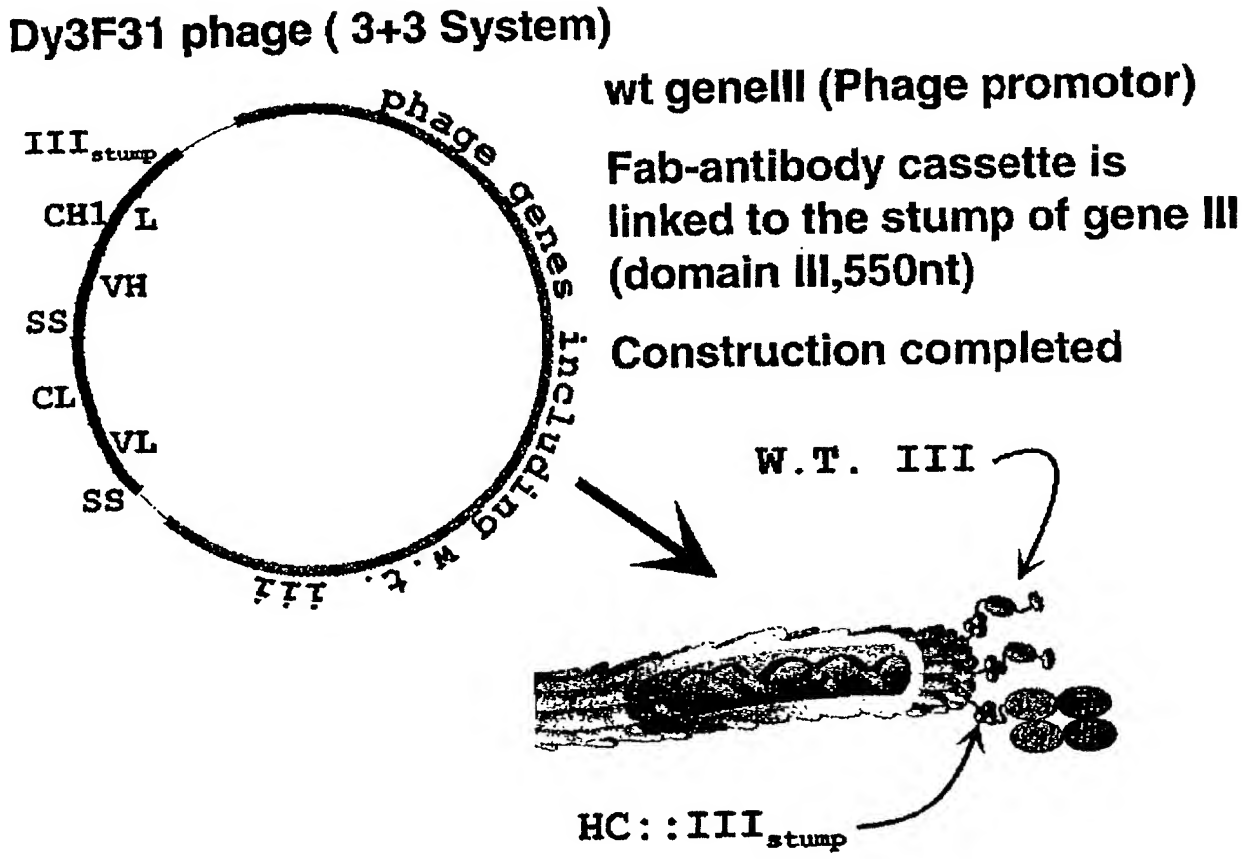
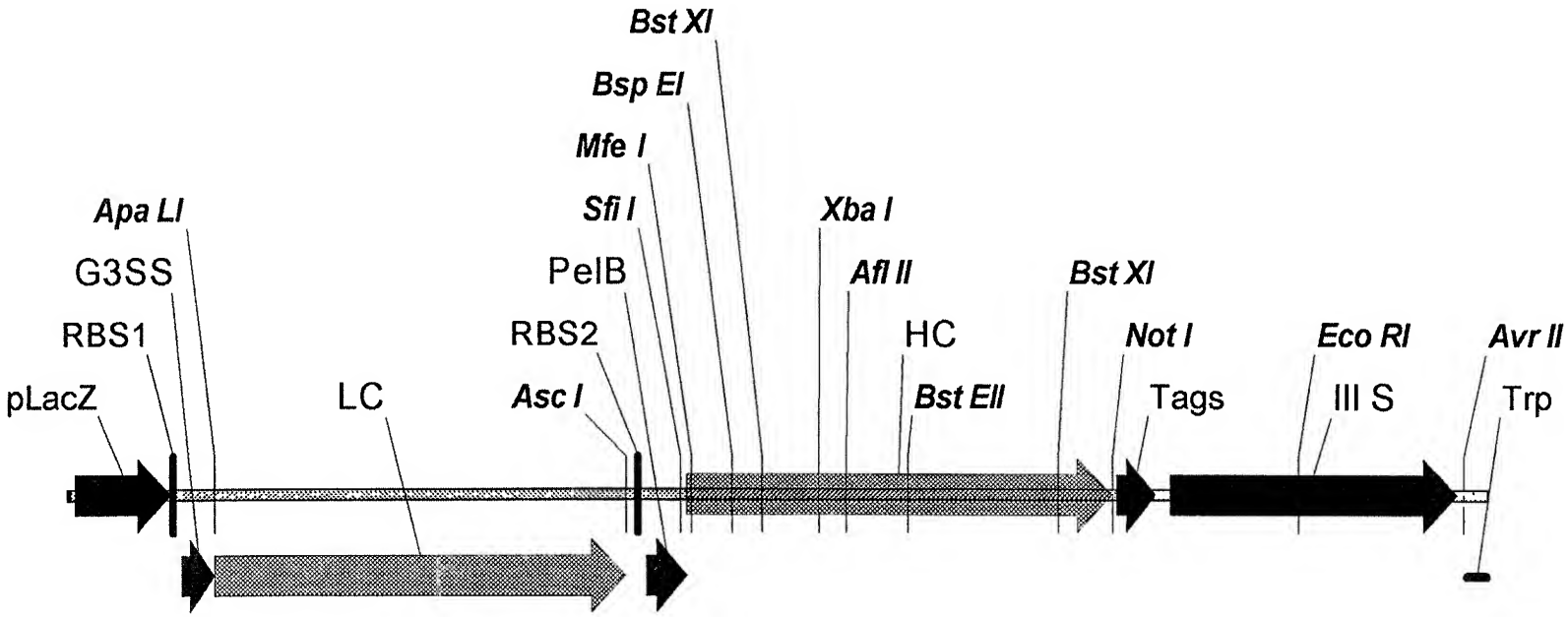


FIG. 16



Fab Cassette

2263 bp

FIG. 17

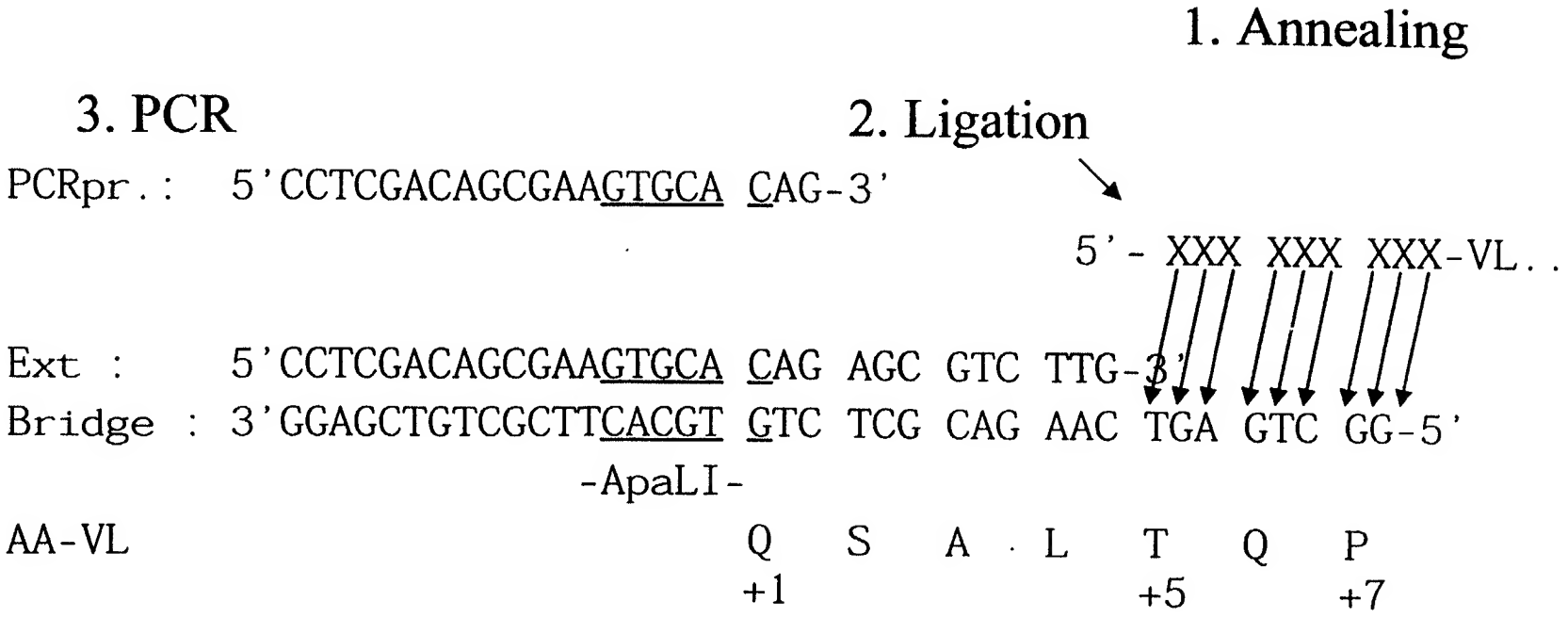

1. Annealing
3. PCR
2. Ligation
PCRpr. : 5'CCTCGACAGCGAAGTGCA CAG-3'
5'- XXX XXX XXX-VL..
Ext : 5'CCTCGACAGCGAAGTGCA CAG AGC GTC TTG-3'
Bridge : 3'GGAGCTGTCGCTTCACGT GTC TCG CAG AAC TGA GTC GG-5'
-ApaLI-
AA-VL Q S A L T Q P
+1 +5 +7

FIG. 18

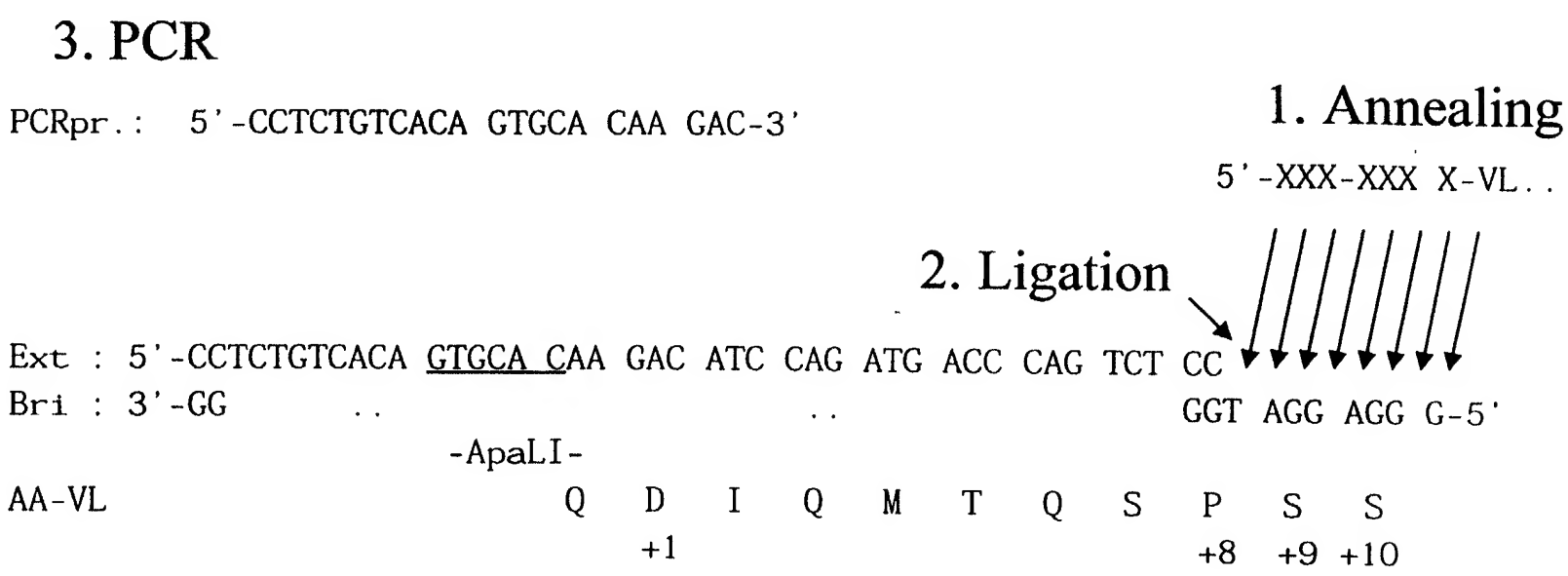


FIG. 19

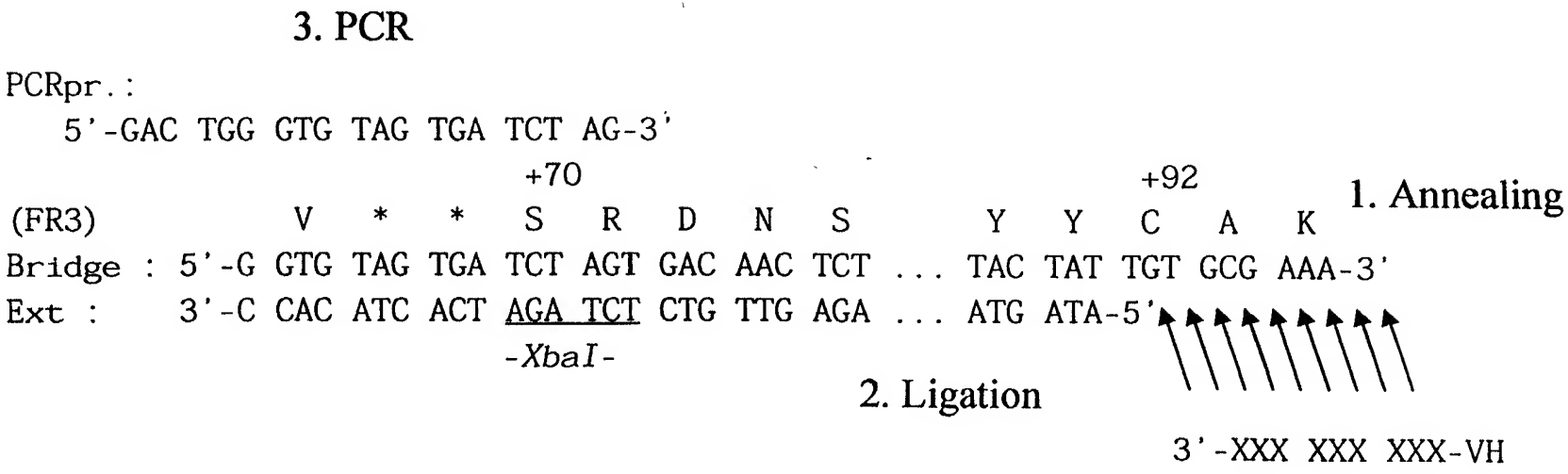


FIG. 20